INHERITANCE IN NICOTIANA TABACUM. II. ON THE EXISTENCE OF GENETICALLY DISTINCT RED-FLOWERING VARIETIES[1]

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IN our studies of inheritance in *Nicotiana Tabacum*[2] it has been demonstrated that the red flower color of *macrophylla* (U. C. B. G. 22/07) is recessive to the light pink of *angustifolia* (U. C. B. G. 68/07), and the same relations are exhibited by the red of *calycina* (U. C. B. G. 110/05) as contrasted with the light pink of *virginica* (Maryland, U. C. B. G. 78/05). In both cases the F_1 was pink, F_2 conformed to the ratio 3 pink:1 red, and F_3 and subsequent generations yielded data consistent with a single factor difference between these two flower colors. It was also shown that when *macrophylla* was crossed with the white-flowering variety *alba* (U. C. B. G. 30/06), F_1 was pink, F_2 conformed to the ratio 9 pink:3 red:4 white, and F_3 and subsequent generations gave data in agreement with a two-factor difference for this character contrast.

Allard,[3] however, had presented evidence, at first sight contradictory to ours, to the effect that the carmine flower

[1] The experimental data cited herein were obtained from cultures made possible by a portion of the Adams' Fund allotted to the Department of Botany by the Department of Agriculture of the University of California.

[2] Setchell, W. A., T. H. Goodspeed and R. E. Clausen, "A Preliminary Note on the Results of Crossing Certain Varieties of *Nicotiana Tabacum*," *Proc. Nat. Acad. Sci.*, 7: 50–56, 1921. A complete illustrated account of these experiments is in press under the title, "Inheritance in *Nicotiana Tabacum*. I. A Report on the Results of Crossing Certain Varieties," *Univ. Calif. Publ. Botany* 5, no. 17. For descriptions and illustrations of the varieties mentioned in this paper cf. Setchell, W. A., "Studies in *Nicotiana*. I., *Univ. Calif. Publ. Botany*, 5: 1–86, 1912.

[3] Allard, H. A., "Some Studies in Blossom Color Inheritance in Tobacco, with Special Reference to *N. sylvestris* and *N. tabacum*." AMER. NATURALIST, 53: 79–84, 1919.

color of his Giant Red-flowering tobacco is dominant to pink in a simple mono-hybrid relation, F_1 being carmine and F_2 3 carmine : 1 pink. He also crossed this carmine-flowering variety with a white-flowering form and obtained light carmine in F_1 and a distribution which might be taken to conform to the ratio 9 carmine : 3 pink : 4 white. We took these results to indicate that his carmine, which must be very similar to our red, was nevertheless genetically distinct from it. This belief was somewhat strengthened by the fact that our red does not fall upon the carmine of the Ridgway[4] color scale, but lies slightly removed from it between rose red and pomegranate purple, although a difference of this kind might conceivably be due to the effect of differences in the residual genotype. We have, however, a variety, *purpurea* (U. C. B. G. 25/06), which exhibits a red flower color somewhat darker and more intense than that of *macrophylla,* and which some preliminary crosses indicated was dominant to pink and white. We accordingly suggested the following factor formulæ for these four colors:

$$WWRRPP = \text{carmine}$$
$$WWRRpp = \text{light pink}$$
$$WWrrpp = \text{red}$$
$$wwRRpp = \text{white}$$

In this formulation *WWRRPP,* represents the basic type, carmine in color; *w,* the difference from it which gives white, irrespective of which members of the pairs occupy the *R* or *P* loci; *p,* that which gives pink; and *r,* that which changes pink to red. Obviously white-flowering varieties may be of four different genotypes, viz., *wwRRPP, wwRRpp, wwrrPP,* and *wwrrpp,* but our white variety *alba* was clearly *wwRRpp.* This formulation brings our results into accord with those of Allard and accounts for the existence of genetically distinct red-flowering varieties. We have now obtained further evidence in support of the correctness of this formulation.

[4] Ridgway, R., "Color Standards and Color Nomenclature," 1912.

We found it necessary to use "Cuba" (U. C. B. G. 200/14),[5] another white-flowering variety, in these studies. Since there is the possibility just indicated of the existence of genetically distinct white-flowering varieties, it became necessary to determine the genetic constitution of "Cuba" with respect to the *Rr* and the *Pp* pairs of allelomorphs. A number of crosses were made, therefore, between "Cuba" and *macrophylla* as the starting point for these determinations. In the account which follows H_{174} = *macrophylla* ♀ × "Cuba" ♂ and H_{175} = the reciprocal. In the season of 1919, 50 plants of F_1H_{174} and 100 plants of F_1H_{175} were grown. They were all pink-flowering except that one plant produced a small white-flowering branch in an inflorescence otherwise pink-flowering. This bud variant, one of the few which we have observed in tobacco, will be taken up in a subsequent report. The further data on these reciprocal hybrids are listed in Table I. The F_2 popula-

TABLE I

F_2 AND BACK-CROSS DATA OF THE CUBA-MACROPHYLLA (WHITE × RED) SERIES

Garden Numbers	Parentage	Flower Color			Totals
		Pink	Red	White	
20.075....	$19F_1H_{174}P_{23}W$	59	22	19	100
20.076....	$19F_1H_{174}P_{23}P$	54	22	23	99
Totals for F_2 populations		113	44	42	199
$16F_1H_{182}$..	$15F_1H_{174}$♀ × 200/14♂	12	—	12	24
$19F_1H_{182}$..	ditto	48	—	50	98
$16F_1H_{183}$..	200/14 ♀ × $15F_1H_{174}$♂	12	—	13	25
$19F_1H_{183}$..	ditto	53	—	47	100
$16F_1H_{188}$..	200/14 ♀ × $15F_1H_{175}$♂	11	—	13	24
$19F_1H_{188}$..	ditto	50	—	49	99
$16F_1H_{189}$..	$15F_1H_{175}$♀ × 200/14♂	6	—	19	25
$19F_1H_{189}$..	ditto	53	—	47	100
Totals for back-crosses to white		245	—	250	495
20.059....	$19F_1H_{174}$♀ × 22/07♂	22	28	—	50

[5] For description cf. Goodspeed, T. H. "Parthenogenesis, Parthenocarpy and Phenospermy in *Nicotiana*," *Univ. Calif. Publ. Botany*, 5: 249–272, 1915.

tions give totals of 113 pink: 44 red: 42 white, whilst the 9: 3: 4 expectation, disregarding fractions, is 112 pink: 37 red: 50 white. In the back-crosses to both the white and the red parents the data are obviously in satisfactory agreement with the 1: 1 expectations. These figures do not establish conclusively the validity of a bigenic formulation for this case, but taken together with the data from the ALBA-MACROPHYLLA series which we have presented elsewhere[6] it seems most reasonable to interpret them in this manner. An alternative mono-hybrid interpretation might be argued, but it would not fit the F_2 totals as well as the dihybrid ratio. The growing of F_3 populations would, of course, soon settle the question, but the results so far secured indicate essential genetical identity of *alba* and "Cuba" in their flower color factors.

In order to demonstrate the difference in behavior of red of *macrophylla* and carmine of *purpurea* we have made parallel crosses between them and a number of other *Tabacum* varieties. The flower colors of these varieties and of their F_1 hybrids with *macrophylla* and *purpurea* are listed in Table II. In each case the F_1 with *macrophylla* was pink but with *purpurea* it was always a full, intense carmine. Among two hundred plants of the CUBA-PURPUREA series one plant appeared which bore

TABLE II

F_1 RESULTS OF PARALLEL CROSSES OF MACROPHYLLA AND PURPUREA WITH A SERIES OF TABACUM VARIETIES

Variety Name and Number	Flower Color	Flower Color of F_1 with Macrophylla	Flower Color of F_1 with Purpurea
angustifolia (U. C. B. G. 68/07).....	Light pink	Pink	Carmine
"Cavala" (U. C. B. G. 72/05).....	Pinkish	Pink	Carmine
"Cuba" (U. C. B. G. 200/14).....	White	Pink	Carmine

carmine flowers on one side and light pink ones on the other. Further studies on this, the most striking case

[6] Setchell, Goodspeed, and Clausen, *loc. cit.*

of somatic variation we have ever observed in *Nicotiana*, are in progress. The F_1 results in themselves sufficiently demonstrate the existence of a genetic difference between the red of *macrophylla* and the carmine of *purpurea*.

We have also secured further data from the CUBA-PURPUREA series which demonstrates the mode of inheritance of carmine when crossed with the same white used in the CUBA-MACROPHYLLA series. These results are set forth in Table III. The totals from the F_2 populations,

TABLE III

F_2 AND BACK-CROSS DATA FOR THE CUBA-PURPUREA (WHITE × CARMINE) SERIES

Garden Numbers	Parentage	Flower Color			Totals
		Carmine	Pink	White	
$19F_2H_{190}$..	$16F_1H_{190}P_8$	58	14	26	98
$19F_2H_{191}$..	$16F_1H_{191}P_6$	28	8	11	47
20.077....	$19F_1H_{191}P_4R$	48	13	39	100
20.078....	$19F_1H_{191}P_4P$	56	13	31	100
Totals for F_2 populations		190	48	107	345
20.060....	200/14 ♀ × $19F_1H_{191}P_4R$ ♂	16	6	28	50
20.061....	200/14 ♀ × $19F_1H_{191}P_4P$ ♂	12	12	25	49
Totals for back-crosses to white		28	18	53	99

190 carmine : 48 pink : 107 white, are to be compared with a 9 : 3 : 4 expectation of 194 carmine : 65 pink : 86 white. The results from the back-crosses, 28 carmine : 18 pink : 53 white, are to be compared with an expectation based on the 1 : 1 : 2 ratio of 25 carmine : 25 pink : 49 white. Pink is again deficient and white in excess, but not to such an extent as to give significance to the figures. Further data from F_3 families would be desirable for completion of the analysis. Thus far the data are in agreement with those presented by Allard for carmine versus pink and white, and they support the conclusion that his carmine variety is identical in its main genetic flower color factors with ours.

The further question now arises as to whether there are any phenotypic differences between carmine and red. There is a detectable difference between the flower color of *macrophylla* and that of *purpurea,* for the former has distinctly more of a purplish tinge and is not quite as intense in coloration as the latter. But these two varieties differ genetically in a large number of other characters. It is not possible, therefore, to decide the question by direct examination, because any distinctions which are found to exist may depend upon differences in the residual genotype rather than upon the specific factor differences which we have studied. Obviously the most satisfactory material for determining the differences between the two colors would be two varieties which had the same residual genotype, but the establishment of such varieties would entail the expenditure of a considerable amount of time and labor. We can, however, obtain some evidence on this problem by comparing the red F_2 segregants of the CUBA-MACROPHYLLA series with the carmine ones from the CUBA-PURPUREA series. In both cases there was a certain amount of variation in intensity of coloration in the F_2 classes, but it was found that, if they were mixed together, it was impossible to separate them again into red and carmine. In cases involving both classes in the same experiment, they would doubtless have to be considered as making up a single phenotype.

We have been interested in determining experimentally whether the morphological similiarities of existing *Tabacum* varieties might safely be taken as an index of phylogenetic affinities. Thus Setchell,[7] commenting on the relationships of *purpurea,* states,

> There are combined in this plant characters of our *N. angustifolia* as to petiole, *N. Tabacum* var. *brasiliensis* as to cucullate tip, tallness, and perhaps also the wing on the petiole, and *N. Tabacum* var. *macrophylla* as to flowers.

It is very natural to regard the sharply constricted leaf-

[7] *Loc. cit.,* p. 11.

base of *purpurea* as a modified petiolate condition, but as a matter of fact our studies have shown that its affinities in this respect lie closer to the sessile leaf type gentically, to which it is recessive, than to the true petiolate class which is dominant to the sessile type. In the present article we show further that the flower colors of *macrophylla* and *purpurea* are distinctly different genetically and their similarity in appearance can not be regarded as an indication of phylogenetic relationship. It is, therefore, evident that any taxonomic system which proposes to portray the phylogenetic affinities of the polymorphic assemblage of *Tabacum* varieties must be derived from genetic studies of character differences.

Allard has suggested the use of these flower color forms for instructional work in genetics. The demonstration of these additional relations increases their interest and value for such purposes. Among other points of interest a cross between *macrophylla* and *purpurea* should give a carmine F_1 and the rather unusual F_2 segregation ratio of 13 carmine (and red) : 3 pink. We have verified the production of carmine F_1 in this cross, but have not yet grown the F_2 progeny. The ease of hybridization, the readiness with which large quantities of guarded seed may be secured, and the extremely long period over which the seed of tobacco retains its viability may be urged as additional advantages in its utilization. Where greenhouse and garden space is available for their growth—plants may easily be grown to maturity in six-inch pots—these varieties and their hybrids would provide excellent material for practise in hybridization and for demonstrations of segregation and unique character interrelations. While there is a certain amount of variation within the several phenotypes here considered, viz., carmine, red, pink, and white, it has not been found to interfere seriously with segregation into the main color classes.